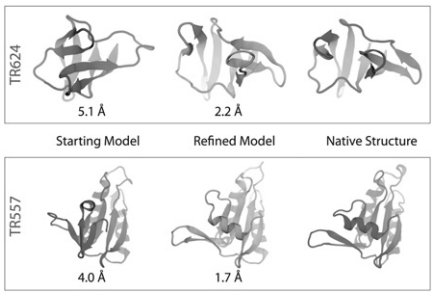
size proteins. We combine this approach with GPU-accelerated molecular dynamics, new implicit solvent models, and recent improvements in force fields. The initial results of this protocol are encouraging and we are able to successfully refine several difficult CASP9 refinement targets.




**3146-Pos Board B7**

**New Developments on Generalized Simulated Annealing Applied to ab-initio Protein Structure Prediction**

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Proteins are the building blocks of cells and the executioners of nearly all cellular functions. Their activity directly depends on their specific three dimensional structure, determined by the folding of its amino acid chain. The folding process ultimately creates a stable structure balancing internal contacts between amino acids and their occlusion to create the protein surface and the hydrophobic core. In this work we explore a new design for applying Generalized Simulated Annealing (GSA) on protein structure prediction, based on previous software developed by our group. The GSA is a stochastic search algorithm employed in energy minimization and used in global optimization problems, such as gravity models, fitting of numerical data and conformation optimization of small molecules. The software deploys a new way of updating the protein structure at each step of the simulation, a different potential energy calculation function based on NAMD and parallel execution of simulations, granting a new take on ab-initio protein structure prediction. The design of the software also allows for the inclusion of data derived from large scale analysis of protein structures from the PDB, as the Solvation Free Energy, allowing us to use information already gathered by experimental structure determinations. We present results on the 20 residue trp-cage mini protein and mastoparan-X, a 13 amino acid peptide. Both chains fold with RMSD of 0,2 nm and 0,1 nm respectively after 10000 GSA steps and a molecular dinamics optimization of 1 ms for trp-cage and 200 ns for mastoparan-X. Structure prediction softwares allow us to study protein structures that cannot be experimentally determined, by using data on chemical bonds, non-bonded interactions and protein solvation. Once approximately predicted, the three dimensional structure can be refined by molecular dynamics simulations.

**3147-Pos Board B8**

**Structure-Energy Relationship of Biological Halogen Bonds: Development of Anisotropic Force Fields**

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Halogen bonds (X-bonds) result from electrostatic attractive interaction between the electropositive crown of a polarized halogen, X, and an electron-rich Lewis base or accepting atom, A, resulting in an X_A distance closer than the sum of traditional van der Waals radii. X-bonds have been shown to direct protein ligand recognition and binding as well as the conformation of biological molecules. We have demonstrated via x-ray crystallography the ability of X-bonds to direct isomeric conformation of DNA Holliday Junctions. The stacked-X junctions can isomerize between two conformations; an X-isomer stabilized by X-bonding at the junction crossover, or the H-isomer stabilized by hydrogen bonding (H-bonding) at the junction crossover. The structures of DNA Holliday junctions incorporating fluorine (F), chlorine (Cl), bromine (Br), or iodine (I) halogenated uracil were determined by single crystal x-ray diffraction from 1.6 to 2.2 Å resolution. Stabilizing X-bonds between the halogenated uracil and phosphate oxygen demonstrate a near linear angle of approach of the oxygen towards the halogen, consistent with current halogen polarization and sigma hole theory. The ratio of each isomer observed in the crystal structure was determined via occupancy refinement calculations. We have shown that this ratio is correlated with the isomeric concentrations present in solution and therefore an indication of stabilization energy provided by either the X- or H-bonding, a conclusion supported by differential scanning calorimetry. We observe that halogen polarization affects both the X-bond structure and strength. The resulting structure and energy relationships of observed X-bonding interactions will be employed in development and parameterization of an anisotropic force field to accurately model the electrostatic and geometric treatment of halogens in current modeling programs. This will facilitate the applications of X-bonding interactions as a tool for biomolecular design and engineering.

**3148-Pos Board B9**

**Absolute Binding Free Energy Calculations to Improve the Accuracy of Near-Native Ligand Pose Predictions**

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When the high-resolution structure of a protein target is available, molecular docking experiments are commonly used for computer-aided drug discovery. Molecular docking experiments are advantageous since they can provide the binding mode of a molecule in a given target protein as well as the binding affinity. However, despite considerable efforts, accurate prediction of ligand poses bound to a target is still challenging due to the protein's structural flexibility. Recent free energy perturbation molecular dynamics simulation (FEP/MD) calculations have shown that the calculated binding free energies are in good agreement with experimental data for co-crystal benchmark targets. Here, we present an integrated methodology in which initial candidate selection from pose decoys obtained by docking is followed by FEP/MD calculations to improve the accuracy of near-native ligand pose prediction. Our approach is evaluated for the small molecule α-helix mimetics inhibiting protein-protein interactions such as p53-MDMX/MDM2 and BAK-MCL-1. The results demonstrate that using the centroid models of the most populated clusters of docking decoys is an efficient approach to select a small set of ligand conformations in which a near-native pose may be included and applying the FEP/MD method enhances ability in discriminating the near-native ligand conformation from the candidates.

**3149-Pos Board B10**

**Structure Based Drug Design in Novel Druggable Pockets on Rho Family GTPases**

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Rho GTPases are conformational switches that control a wide variety of signaling pathways critical for eukaryotic cell development and proliferation. They represent attractive targets for drug design as their aberrant function and up-regulation is associated with many human diseases including cancer. Extensive high-resolution structures and mutagenesis studies have laid the foundation for the design of new structure-based chemotherapeutic strategies. We describe the application of molecular dynamics (MD) simulations, principal component analysis, sequence conservation analysis, fragment mapping and ensemble small-molecule docking to provide a complete mapping of potential small-molecule binding pockets.

Characterized sites include novel pockets in the vicinity of conformationaly responsive switch regions as well as distal sites that appear to be allosterically linked to the nucleotide and effecter binding regions. Intensive virtual screening and docking calculations applied to these specific novel sites revealed promising compounds candidates for experimental assays. Furthermore, the use of a single accelerated MD simulation, advanced MD method that extends the accessible time-scale of conventional simulations, reveals a distribution of binding sites equivalent to the sum of the accumulated crystal structures including transient binding sites that are practically impossible to observe with conventional MD.

**3150-Pos Board B11**

**Investigating the Mechanism of Activation and Inhibition of the Phosphate Dependent Mitochondrial Glutaminase**

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Glutamine metabolism often plays a crucial role in the metabolism of cancer cells that exhibit high rates of aerobic glycolysis. The enzyme primarily responsible for the conversion of glutamine to glutamate, and ultimately the rate of α-ketoglutarate flux into the citric acid cycle, is the mitochondrial enzyme glutaminase. The fully processed form of the human enzyme can be expressed in bacteria, is fully active, and in this study we use a combination of

techniques including MALS (Multi-Angle Light Schattering), X-ray crystallographyic, and SAXS (Small Angle X-ray Scattering) to better understand the nature of the active conformation of GAC and ultimately, how the recently described glutaminase inhibitor 968 (J.B., Wang; Cancer Cell, 2010) interacts with GAC and renders it inactive. Our results support a model whereby GAC binds phosphate that results in a change in glutaminase oligomerization, which results in active tetramers as well as higher oligomeric species. Effects of the drug 968 on the activation/oligomerization state of glutaminase will also be discussed.

**3151-Pos Board B12**

**A Systematic Computational Method to Predict and Enhance Antibody-Antigen Binding in the Absence of Antibody Crystal Structures**

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Structure-based design efforts on antibodies are frequently hamstrung by the general difficulty of crystallizing antibody molecules. Computational approaches are getting more success and playing a very important role in protein structure prediction and design. A combined platform with both computational predication and experimental verification would have a transformative research capability on antibody engineering. We have been developing and integrating RosettaAntibody (homology modeling and H3 loop modeling), RosettaDock (SnugDock and EnsembleDock) and RosettaDesign (non-canonical amino acids design) into a systematic computational pipeline. Using the targets of M18/Anthrax complex, which has solved crystal structure, and anti-MS2/MS2 complex, whose antibody structure is not available, the new pipeline is capable of building homology models for antibody from its sequence, and predicting the docked antibody-antigen structures. The correct antigen epitope can be identified by iteratively refining the predicted candidates with experimental verifications such as interface alanine scanning. This is followed by computational CDR designs utilizing non-canonical amino acids, which can form covalent bonds with epitope residues. Along with experimental confirmations, the incorporation of the systematical computational method was proved to significantly facilitate the epitope identification and non-canonical amino acid designs. These efforts eventually substantially increased the antibody-antigen binding affinity of the two targets.

**3152-Pos Board B13**

**A Molecular Dynamics Simulation of Peptide-Triazole HIV Entry Inhibitor Binding to gp120 Hydrophobic Core**

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The envelope spike on HIV surface is a non-covalent trimer of gp120 and gp41 dimers; with gp120 responsible for binding to the CD4 receptor and the mandatory coreceptor, CCR5 or CXCR4. Blocking the gp120-CD4 or gp120-coreceptor interaction can block viral entry and infection. The HNG family of peptides (sequence RINNIXWSEAMM, X= derivatized azidoproline) is a promising class of dual-site entry inhibitors thought to allosterically inhibit the binding process and trap the flexible gp120 molecule in an inactive state. To date, there have been no reports on the structure of the peptide or the peptide/gp120 complex. Here we have used Molecular Dynamics to develop a docking model in which the peptide binds in a tripartite fashion, preventing the formation of the bridging sheet in gp120, which is necessary for coreceptor attachment and initiation of entry. The protein undergoes significant induced fit conformational changes involving movement of large loops which allows the peptide to bind tightly. Our model is consistent with experimental observations on the peptide footprint on gp120, pointing to a hydrophobic core underneath the bridging sheet and close to the F43 pocket as the putative binding site. Our results provide an explanation for why the stereochemistry of the triazole moiety on the proline is important for peptide function, in addition to explaining the inactivity of D-tryptophan in the sequence. Furthermore, saturation transfer difference (STD) NMR experiments point to the I-X-P hydrophobic center on the peptide as the central contact point in the complex, which is consistent with the peptide pose proposed in MD studies. Our model may provide a unique basis for rational design of allosteric entry inhibitors of HIV and ultimately small molecule inhibitors of viral entry.

**3153-Pos Board B14**

**Evaluating the Potency of Small Molecules to Combat Resistance Based on Docking Structures: An Application to the HIV Protease**

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HIV-1 protease is an important drug target for the therapy of AIDS. Up to date nine protease drugs have been approved by the U.S. Food and Drug Administration. However, the efficacy of these drugs is limited by the drug-resistant mutants of the protease which can become dominant in the viral population in several months. As it often takes years of effort and millions of dollars to develop a drug, evaluating the potency of a compound to combat resistance at the early stage of drug development would is no doubt invaluable. In this study, we present a new procedure to predict HIV protease mutants that can be resistant to a specific compounds based on docking structure. We exploited molecular interaction energy components (MIECs) between the ligand and the protease residues to characterize the docking poses. A classification method called Support Vector Machine (SVM) is then employed to distinguish resistant from non-resistant mutants. Most interestingly, leave-one-drug-out test showed that our strategy can be generalized to evaluate potency of new drug lead, which suggests the possibility of evaluating the potency to combat resistance for small molecules found from virtual screening.

**3154-Pos Board B15**

**Reconstituted High Density Lipoprotein Bearing Apolipoprotein E3 Serves as a Nanovehicle to Transport and Target Bioflavonoids such as Resveratrol**

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The objective of this study is to transport resveratrol, an antioxidant and anti-inflammatory bioflavonoid, in the plasma to targeted sites. Resveratrol is a phytoalexin polyphenol that is found in natural products such as grape skins, cocoa, and is a constituent of red wine. It appears to be a potent factor as an anti-cancer and atheroprotective agent. The ''French Paradox'' (French suffering a relatively lower incidence of coronary heart disease compared to other western societies) has been attributed to the benefits of resveratrol (as a result of higher consumption of red wine by the French). However, it is difficult to achieve effective concentrations of resveratrol in plasma via the oral tract from natural resveratrol-rich products. Its low aqueous solubility appears to limit the use of resveratrol as a nutraceutical agent. We propose to employ apolipoprotein E3 (apoE3)-containing reconstituted high density lipoproteins (rHDL) for transportation and targeted delivery of resveratrol. rHDL are water soluble lipoprotein complexes composed of a bilayer of phospholipids surrounded by apoE3, which bears high affinity binding sites for the cell surface localized low density lipoprotein receptor (LDLr). The phospholipid microenvironment of rHDL is expected to provide a centrally located lipid milieu into which resveratrol may be sequestered, and shielded from modification and/or degradation. Our data indicates that resveratrol has effectively partitioned into the hydrophobic milieu of the phospholipid bilayer of rHDL without significant alteration to the conformation of apoE3. Importantly, the presence of resveratrol did not alter the LDLr binding ability of apoE3. These results indicate that rHDL bearing the apoE3 LDLr binding domain can serve as an effective ''nanovehicle'' to transport and potentially deliver resveratrol to targeted sites across the cell membrane.

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**3155-Pos Board B16**

**Design of An Ultra-Fast Acting Insulin Analog using Unnatural Amino Acids - Replacement of PheB24 with Cyclohexanylalanine**

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Insulin replacement therapy is central to the treatment of Type 1 diabetes mellitus (T1DM). Clinical trials have shown that tight glycemic control in T1DM leads to reduction in long-term diabetic complications. In an effort to better mimic the normal post-prandial pattern of pancreatic insulin release, mealtime insulin analogs were developed in the 1990s using standard mutagenesis. These analogs exhibit more rapid absorption from the subcutaneous depot due to destabilization of the zinc insulin hexamer. Unfortunately, such conventional analogs do not achieve the rapid ''on-off'' pharmacodynamic profiles sought in an ideal rapid-acting insulin formulation. To circumvent this limitation, we have investigated the utility of nonstandard protein design based on unnatural mutagenesis. Our studies have focused on residue PheB24 at the dimer interface of the insulin hexamer. Investigation of the role of this aromatic side chain by hybrid quantum-mechanical/classical molecular mechanics (QM/cMM) suggested that substitution of the B24 aromatic ring of its saturated